

# Research Progress Log

The research log is a document where we tracked our daily progress. It includes descriptions about our main tasks and goals throughout the year. Most of the daily progress of prototype experiments or research done before summer around January to April are listed here.

(Only Tasks/Topics/To-Do's -- the responses & works should be on the other docs or Lab notebook)

Key: **Priority/Urgent**

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## 4/28/2016 B5 Log

Experimental	Human Practice	Prototype/Research/Modeling
<ul style="list-style-type: none"><li>• Digested BBa_K880005 and GFP (For remaking the GFP His</li><li>• (Digested His Term Part need to be found)</li><li>• Ran PCR Check for BBa_CRYAA</li><li>• PCR Clean up of GSR w/ restriction sites<ul style="list-style-type: none"><li>○ Only a segment, don't be surprised by the concentration</li></ul></li><li>•</li></ul>		<ul style="list-style-type: none"><li>•</li></ul>
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## 4/27/2016 A5 Log

Experimental	Human Practice	Prototype/Research/Modeling
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<ul style="list-style-type: none"> <li>Continue fish lens experiment from the new lens that we got yesterday</li> </ul>	<p>Marketing: start working on 4Ps and separate documents created. We will keep working on make more decisions for our product and research on sample market plans to organize all the information into actual one document plan.</p>	<ul style="list-style-type: none"> <li>A new paper has been found that can possibly tell us more about how the nanoparticles travel into the lens.</li> <li>Another paper that is more math-based actually has a bunch of equations and math models that tells us how nanoparticles interact with cell membranes.</li> </ul>
	<p>Discussed stuff about videos</p>	<p><a href="#">WDeadlines for the Next 4 Weeks for Modeling/Research:</a></p> <hr/> <p>By...</p> <ol style="list-style-type: none"> <li>4/31 Lanosterol</li> <li>5/9 Finalize all Nanoparticle Research</li> <li>5/20 Finalize all graphs and functions for GSH/GSR/H2O2 effects</li> <li>5/27 Finish Modeling Nanoparticles delivery</li> </ol>
	<p>May 2nd, 2:30 we have a meeting with a vet.</p>	

#### 4/27/2016 A5 Log

Experimental	Human Practice	Prototype/Research/Modeling
<ul style="list-style-type: none"> <li>Bought a new fish</li> <li>Dissected the fish eye, isolated the fish lens, and left it in sealed tube with saline solution (filled <math>\frac{3}{4}</math> of the way).</li> <li>The saline + lens is then taped onto the shaker and left for overnight incubation.</li> </ul>	<p>Further edited the letter for cataracts day</p> <p>Dr. Kuo</p>	<p>Avery will be combining two sets of data that he has compiled, to get a graph for the relationship between (basically) H2O2 and GSH activity</p>

	Discussed stuff about videos	<p>WDeadlines for the Next 4 Weeks for Modeling/Research:</p> <hr/> <p>By...</p> <ol style="list-style-type: none"> <li>6. 4/31 Lanosterol</li> <li>7. 5/9 Finalize all Nanoparticle Research</li> <li>8. 5/20 Finalize all graphs and functions for GSH/GSR/H2O2 effects</li> <li>9. 5/27 Finish Modeling Nanoparticles delivery</li> </ol>
	May 2nd, 2:30 we have a meeting with a vet.	

#### 4/26/2016 B5 Log

Experimental	Human Practice	Prototype/Research/Modeling
Finished collecting 94 hour point for cataract model	Called Dr. Kuo and two hospitals, worked on the questions we're asking them	Looking into more information about the general eye structure in order to better understand the course of nanoparticle traveling
	Continued working on Marketing Plan	
	Dr. Kuo replied. She suggested that we see what time we are free, and then get back to her again (before April 29th) to decide when we want to meet for an interview). Then, she'll talk to her hospital director about whether we can watch her performing a cataract surgery We probably need to sign a confidentiality form (or somethign like that)	

#### 4/25/2016 A5 Log

Experimental	Human Practice	Prototype/Research/Modeling
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<ul style="list-style-type: none"> <li>The SEM images of Nanoparticles (taken on Friday 4/22/16)</li> <li>Lens Testing</li> </ul>	<ul style="list-style-type: none"> <li>Editing Letter for National Cataracts Awareness Day</li> <li>Emails/Contacts: <ul style="list-style-type: none"> <li>Avery needs to ask his dad about an official appointment</li> <li>Need to call Dr. Kuo (郭雅慧) M.D. @ Evergreen General Hospital for an appointment</li> </ul> </li> <li>Survey-- EVERYONE NEEDS TO ASK THEIR PARENTS TO FILL OUT THE SURVEY!</li> </ul>	<ul style="list-style-type: none"> <li>The first graph has been created! <ul style="list-style-type: none"> <li>Shows H<sub>2</sub>O<sub>2</sub> concentration vs. GSH Redox Potential</li> <li>Thanks Avery!</li> </ul> </li> </ul>
		<p>Deadlines for the Next 4 Weeks for Modeling/Research:</p> <hr/> <p>By...</p> <ul style="list-style-type: none"> <li>10. 4/31 Lanosterol</li> <li>11. 5/9 Finalize all Nanoparticle Research</li> <li>12. 5/20 Finalize all graphs and functions for GSH/GSR/H<sub>2</sub>O<sub>2</sub> effects</li> <li>13. 5/27 Finish Modeling Nanoparticles delivery</li> </ul>

#### 4/15/2016 A5 Log

Experimental	Human Practice	Prototype/Research/Modeling
<ul style="list-style-type: none"> <li>Miniprep CRYAA (with terminator)</li> <li>Was supposed to miniprep CH25H → but there was no bacterial growth (probably mixed up the antibiotics!)</li> <li>Have already</li> </ul>	<ul style="list-style-type: none"> <li>Finalized the cheat sheet for all iGEM people to use as a reference</li> <li>(esp. During Spring Fair, when answering customer's questions)</li> </ul>	<p>Continuing Modeling Research</p> <p>Need to Discuss with Avery regarding the "big picture"</p> <ul style="list-style-type: none"> <li>Need to reanalyzer to prioritize what's to be done in the next few weeks</li> </ul>

<ul style="list-style-type: none"> <li>Prepared a chitosan solution and tpp using a brand new protocol, in the fridge 4C</li> </ul>		

#### 4/14/2016 B5 Log

Experimental	Human Practice	Prototype/Research/Modeling
Ran SDS page for fish and pig lenses	<p>Created Posters for Spring Fair</p> <ul style="list-style-type: none"> <li>Posters explaining the organization to which we're donating (Himalayan Cataracts Projects @ cureblindness.com!!!)</li> <li>A day please help us finish up the poster by adding the info about the charity!</li> </ul>	Found the lanosterol Concentration
	In progress of making cheat sheet for our entire project--the research/experimental plan, the human practice, the wiki... all of it	
	<p>ALSO please check that all the ipad works - load the survey and try to submit the survey with the lab ipads!</p> <p>ANYONE WHO HAS A SPARE IPAD TO USE PLEASE BRING THEM ON SAT, if you have Yoga and you can bring it please also do so during your shift. (for piano tile)</p>	

#### 4/13/2016 B5 Log

Experimental	Human Practice	Prototype/Research/Modeling
PCR GSR (start cloning cycle)	<p>Taught 7th Grade! :D Had lots of fun</p> <p>Finished baking cupcakes!! And fudge!!</p>	
<p>Used the reagents from B5 and refiltered Chitosan through 0.45 um filters. Did not gelify</p> <p>Checked under microscope for</p>		

some scarce signs of nanoparticles (was there)		
Gel Checked Prom-GFP-His-Term, the gel was very blurred, probably due to the extended time of the gel electrophoresis		

#### **4/12/2016 B5 Log**

<b>Experimental</b>	<b>Human Practice</b>	<b>Prototype/Research/Modeling</b>
PCR GSR (start cloning cycle)	Taught 7th Grade! :D Had lots of fun  Finished baking cupcakes!! And fudge!!	
Used the reagents from B5 and refiltered Chitosan through 0.45 um filters. Did not gelify  Checked under microscope for some scarce signs of nanoparticles (was there)		
Gel Checked Prom-GFP-His-Term, the gel was very blurred, probably due to the extended time of the gel electrophoresis		

#### **4/12/2016 B5 Log**

<b>Experimental</b>	<b>Human Practice</b>	<b>Prototype/Research/Modeling</b>
Started another round of Chitosan nanoparticles. Didn't gelify again. There is about 5 ml of Filtered Chitosan remaining in the 4c fridge	Taught 7th Grade! :D Had lots of fun	
PCR Check for Transformation of BBa_GFP_HIS_B0015		

Jason and Changsun ate a Cupcake		
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#### **4/11/2016 A5 Log**

Experimental	Human Practice	Prototype/Research/Modeling
<p>Started another round of Chitosan nanoparticles, previous don't seem to have worked. Didn't gelify</p> <p>Mixing Chitosan particles for 24 hours</p>	<p>Finished worksheet</p> <p>Prepped for 7th grade presentation</p> <p>Started on cheatsheet for spring fair and sale</p>	
Prepared cell culture of BBa-GFP-His, take out tomorrow		

#### **4/8/2016 B5 Log**

Experimental	Human Practice	Prototype/Research/Modeling
Mixed aqueous Chitosan diluted solution and TTP to make CS/TPP nanoparticle solution		
Ligated and Transformed CRYAA into B0015 Ig em BioBrick		

#### **4/7/2016 A5 Log**

Experimental	Human Practice	Prototype/Research/Modeling
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	Finished translating document	
	<p>Tested running gels for 7th grade. Found out that some gels don't work so we are making more (more still need to be made). Found out that one of the gel boxes doesn't work so we need to test all of them and label the ones that work.</p> <p>On a positive note we confirmed that the yellow colour is positively charged and the blue is negative.</p>	

#### 4/6/2016B5 Log

Experimental	Human Practice	Prototype/Research/Modeling
Digestion for promoter + rbs + GFP + His & B0015	<p>A day please confirm details for baking - when and where.</p> <p>Everyone signed up for iGEM teams</p>	Read a paper on h202 induced cataracts model for pig and rat
Prepared Liquid Reagent for Solid Chitosan (By Hansen)	Modified and finalized spring fair survey and games. Megan will bring two iPADS	
	Assigned people for 7th grade outline, also stuck on the board.	

#### 4/5/2016 A5 Log

Experimental	Human Practice	Prototype/Research/Modeling
Started planning out the chitosan nanoparticles experiment, Created the TPP solution	checked the 7th plan and handwritten a outline (red ink, stick on the board) B day leaders sign up names on the category u want!	
	revised the plan abt the dry lab edited the intro video - cut down to 3mins	



	translated spring fair survey	
	fundraising - we need to decide how to pre-bake cupcakes and how to decorate	
	<b>KEY DATES NEXT WEEK: TUES+WED period 4+5 7TH GRADE THURS flex + lunches CUPCAKE SALE SATURDAY SPRING FAIR BOOTH</b>	

### 3/25/2016 B5 Log

Experimental	Human Practice	Prototype/Research/Modeling
Protein gel check - looking good but still need to find the specific size for alpha beta gamma crystalline in fish lenses. Photos uploaded on gdrive	Made props for spring fair! Covered glasses with styrofoam	Continued on modeling research: found concentration for 25 HC. Still need to find GSR and GPX concentration in normal and lens with cataracts
Miniprepmed promoter + gfp + his (A DAY please nanodrop, couldn't do it because the old pipettes aren't accurate)	Decided on the games for spring fair	
PCR check of promoter + rbs + gfp + his was good ~1k bp (need confirmation)	Market research ongoing	
Dissected 4 fishes. Stored 7 lenses w/o water in the freezer	Sent out email asking about animal model	
Dissected another two pig eyes. Lens are in the freezer as well		

### 3/24/2016 A5 Log

Experimental	Human Practice	Prototype/Research/Modeling
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PCR BBA_GFP_10x His Tag mixture	Finalizing the organization we are sending our fundraising money to (. Started discussing baking, decided on cupcakes with fondant for icing (eyeball shaped).	Still looking for more information about the fish lens structure
Transformed CH25H into Amp Agar Plates	Need to work on Spring Fair stuff, that should be prioritized.	-- need to figure out whether the soft outer layer of the fish lens is actually the cortex, or just a protective layer (if so, what is it called?)
	Further specified 7th grade plan (on the second document with the time frame). Concrete script is nearly done. There are still a few materials to make sure we have (gel boxes, pipettes, filling multiple centrifuge tubes with dye).	
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### 3/23/2016 B5 Log

Experimental	Human Practice	Prototype/Research/Modeling
Transformed BBA_K880005 + GFP & HIs-tag	Fundraising -- communicated with Zach regarding the fundraising ideas (?)	Still looking for more information about the fish lens structure
Transformed CH25H DNA  DNA stored in the Counteracts box	Finished 7th grade art stuff	-- need to figure out whether the soft outer layer of the fish lens is actually the cortex, or just a protective layer (if so, what is it called?)
Liquid Culture for CRYAA (already in iGEM biobrick Backbone) -> Has to be MINIPREPPED SPECIFICALLY FOR SEQUENCING ( <b>ASK Andrew for specific procedure for sequence minprepping</b> )	Did some work (made some decisions) on marketing plan - we need stats of approx how many people for each market group	
Fish Lens Experiment Created protocol for the experiment, biuret test w three groups 1) ddh20 2)		

nucleus 3) cortex - need to do the experiment again, this time with the rest of the 16 lenses - Tomorrow run protein gel and test 5 groups		
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### 3/22/2016 A5 Log

Experimental	Human Practice	Prototype/Research/Modeling
Ligated GFP/10x His Tag + BBaK880005  Collected it and put it back into fridge  Transformation tomorrow	Transcribed Doctor Moran's interview	Modeling: Found Antioxidant Activity (GSH and catalase) vs. H <sub>2</sub> O <sub>2</sub> concentration model.  Mathematical equations found (see Modeling papers).  Will find relationship between age and GSH activity.
	Nearly finished 7th grade arts and crafts, only a few more cut outs left.	

### 3/21/2016 B5 Log

Experimental	Human Practice	Prototype/Research
Dissected 6 fish eyes (there are 10 more in the bio storeroom) - Fish eye in ddh20 - Lens in ddh20 - Lens in 50 uM h202 - Lens in 500 uM h202 start tmr: - Lens under UV for 1 hr - Lens under UV for 6 hrs	Pretty much done with transcribing Mrs. Clapper's interview. Also worked on transcribing Dr. Moran's interview.	Found the GSH concentration in the lens

	Worked on the construct parts for 7th grade workshop	
	Did market research on competitors	
	Sent out emails asking about protein eyedrops	

### 3/18/2016 A5 Log

Experimental	Human Practice	Prototype/Research
Did Digestion for GFP-10x His Tag using the previous mini-prep ~211.7 ng/microLiter	Received email response from 高醫大團隊的卓教授 , need to ask him more specific question, ms.chiang suggests that the person who sent the email + did the research on this team should be the one responding to email	Found two graphs linking the relationship between... 1) Age vs. crystallin function 2) Age vs. oxidative damage
Finished 4 tubes of Miniprep (OF WHAT?!?!?!?!? - Chiang)  Didn't not test concentrations (didn't have time)	Had a mental breakdown, but took it easy.  Started working more on the 7th grade interactive gene workshop.	Found links about H2O2 HOWEVER, still need to find (preferably) a graph showing correlation between H2O2 accumulation and age
	Made 26 gels.	Will also need to look into more stats about nanoparticles 1. Size 2. Concentration (appropriate for our eyedrop)
	Halfway through transcribing the Dr. Moran interview.	
	Worked some more on cupcakes.	

### 3/17/2016 B5 Log

Experimental	Human Practice	Prototype/Research
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	<p>Made 6 small gels for 7th grade (no seeing safe!) Please take out (store separately) &amp; make more :)</p> <p>Uploaded the audio for Dr. Moran's interview!</p>	
	<p>tested and compiled all the dyes for 7th grade (labeled + group + sealed them in bag)</p>	
	<p>organized the paper construct parts - need to continue to make more parts :) yay art!</p>	
	<p>reply back to 博士倫 saying thanks and get back when needed!</p>	
	<p>Talked with Mrs. Bruce about marketing. We need to start with narrow down the market groups (planning file is created) and details about what is in a marketing plan is also in drive so go take a look! Edited the survey questions for spring fair (translating still in progress)</p>	

### **3/16/2016 A5 Log**

<b>Experimental</b>	<b>Human Practice</b>	<b>Prototype/Research</b>
<p>Did Mini-Prep on GFP-10x His Tag, newly ligated and transformed from liquid culture</p>	<p>Emailed Mr. Brocklehurst explaining new plan (Angela should receive email). New plan is to have a brief intro, go into a gel exercises in groups of 5-6, and then do the interactive gene construct exercises in the small groups. Ask Mr. Clapper for specifics.</p>	
<p>Also re did PCR on GFP-10x His Tag from Colony/Plate 3 so Gel Check again today</p>	<p>Sent an email to Bosche, they said they can't help us again.</p>	
	<p>Called a company and they will come to help answer our questions next week.</p>	
	<p>Started final plan for 7th grade</p>	

### 3/15/2016 B5 Log

Experimental	Human Practice	Prototype/Research
Ligating CRYAA with BBa-k88005  Transforming the ligated plasmid into competent cells  PCR -- Run - of ... of GFP + 10xHis-Tag	<ul style="list-style-type: none"><li>Sent email back to Basch with our questions and proposal</li></ul>	Need to order these: GSH (confirmed) 25 HC Lanosterol Cholesterol TPP
Isolate Fish Lens One in the UV Illuminator -- under UV for 30 minutes Another in room temperature	<ul style="list-style-type: none"><li>Prepare the construct components (art time lol)</li></ul>	TO DO: Look into how to make lenses cataractous <ul style="list-style-type: none"><li>Boil lens</li><li>Sonicate</li><li>Treat with H2O2</li><li>UV light</li></ul>
	<ul style="list-style-type: none"><li>Sent email to professor who led a team to develop eyedrop for nearsightedness</li></ul>	TO DO: 1) Find concentration of H2O2 in lens 2) Find GSH and GSX concentration in our lens
	<ul style="list-style-type: none"><li>Ms. Bruce coming by B5 Thursday to introduce marketing plan</li></ul>	TO DO: Find equations for modeling <ul style="list-style-type: none"><li>1) H2O2 vs. CryAA and CryAB</li><li>2) H2O2 vs. GSH</li></ul>
		Looked up internal conditions within the fish. Need to confirm whether or not it is similar to mammalian lenses.

### 3/14/2016 A5 Log

Experimental	Human Practice	Prototype/Research
Checked that CryAA was in box  Started PCR for 10x His Tag - GFP ligated product plus a negative control of purified vector (10x His Tag + backbone)	<ul style="list-style-type: none"><li>IBSL and Dr. Wu interview success</li></ul>	<ul style="list-style-type: none"><li>Finalized plan for testing</li><li>FOR NEXT CLASS:<ul style="list-style-type: none"><li>Research for concentrations (onenote)</li><li>CRYAA and CRYAB dimerization</li><li>Order things</li></ul></li><li>Confirm that the primers has been sent</li></ul>

BDay will need to do Gel Check	Finished working on some stickers.	
	Took some photos of experimentation. Created a template email proposal for all companies.	
	Worked on planning for Kindergarten Tomorrow. Got nearly all materials ready.	
	Finished translating email from vet.	

### **3/11/2016 B5 Log**

<b>Experimental</b>	<b>Human Practice</b>	<b>Prototype/Research</b>
Ran gel for CRYAA Insert and BBa_K880005 vector and purified them  PUrified gel Stored in 4c "Igem exp. in progress" box"	Can someone give Angela the snapchat account password thanks LOL	DECIDED TO WORK WITH 25 HC!
Ligation+Transformation for 10x Histidine tag vector and GFP Insert  Should incubated overnight and taken out 3/12	Interviewed Mrs. Clapper YAY :) video recorded and got information about people go through cataracts surgery and how cataracts influence their lifestyle	
	Worked on the 7th grade dry lab activity (cut out sample parts in color paper)	
	Gel activity trials - different dye	
	sent out 7 email to eye drop and contacts manufacturers - got one response from 博士倫 saying they wanna work with us!!!!!!!!!! YAY	

### **3/10/2016 A5 Log**

<b>Experimental</b>	<b>Human Practice</b>	<b>Prototype/Research</b>
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Finished Gel Extraction for 10x His Tag * 2/GFP *1/BBa_K880005*1 (Did not have time to test the concentration)	Created a snap chat account: TASiGEM2016	Looked into 25HC to confirm its function in restoring solubility of the proteins and reversing aggregation
Purified PCR'ed EX_CRYAA_SP DNA	Made T-Shirt and started making a donation sticker	
Digested CRYAA PCR Clean up sample and BBa_k880005 to put CRYAA insert into BBa_K880005 vector igem biobrick backbone	Worked on the 7th grade gel activity, tried out some different dyes (if you want to do more with this talk to Dr. Chiang).	
	<b>Found several potential fundraising ideas including cupcakes</b>	
	sent out 9 email to manufacturers	

### 3/9/2016 B5 Log

Experimental	Human Practice	Prototype/Research
Digested BBa_k880005	Finish up 7th grade presentation - filming explanatory video clips + finalize slides + finalize the plans for the 4 labs (3 wet + 1 dry)  - We Made a tutorial video on mini prepping for Kids. Presentation is almost complete. Video needs to be edited now. Also narration (maybe)	Verified that SKQ1 is a chemical compound
Ran gel for + cut out GFP & 10xHis-Tag	Sent email to Mr. Brocklehurst to confirm how the sessions will run.	Reminder: Finalized our treatment by this Friday
	Sent email to Dr. Moran for interview inquiry.	
	<b>Spring fair - we need to do more on this! finalize which games (what to buy &amp; prep &amp; make) / what merchandises (use which designs) / SURVEY!!!!!! / others</b>	



	IBSL bio class presentation	
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### 3/8/2016 A5 Log - **A DAY PLEASE UPDATE LOG for HP and Prototype!!!!!!**

Experimental	Human Practice	Prototype/Research
Purified PCR CRYAA Also: Set up digestion for histag (EX) and GFP (SP)		
What needs to be done: tPCR CRYAA -- we need to rerun this because last time hte		

### 3/7/2016 B5 Log

Experimental	Human Practice	Prototype/Research
(Update from Friday PD Day) Gel Check for GSR verified that the digestion of GSR was a <u>success</u> !	7th grade plan finalized - we decided on doing just 4 activities for the first lesson because we only have 60 mins of time with them. Other activities/ideas can be done in future lessons.	Looked into other types of treatments: SKQ1 Eye drop and the three amino acid compounds - SKQ1 is found to reverse aggregation <u>and</u>
1. Purified PCR CRYAA 2. Set up digestion for histag (EX) GFP (SP)	Reviewed the email response from one of the local pets (email content in google drive for people who wanna talk a look)	Checked the GSR reverse primer sequence sent back from Tri-I, GSR sequence is confirmed.
	Spring fair more ideas + examples	Still need to look into different types of nanoparticles

**3/2/2016 A5 Log**

Experimental	Human Practice	Prototype/Research	Biobuilder Poster
	Worked further on concrete plan for 7th grade, added new idea about changing the pipetting and gel workshop. Created concrete problems for them to solve with different gene constructs	Found that Chitosan particles are taken into epithelial cells via active transport  Are well tolerated by ocular surface tissue, does not disrupt cell viability	
		GSR PCR check	
	Finished translating pet organization email.		

**3/1/2016 B5 Log**

Experimental	Human Practice	Prototype/Research	Biobuilder Poster
	sent out more emails - vet/professors	looked into PECE nanoparticles, refer to google doc	DONE!! :-)
	more brainstorm on spring fair games - need more ideas and planning	Checked the ORF and designed forward and reverse primers for CH25H	
	start planning on meeting with blind school students - need more concrete planning!!!	Looked into the efficiency for ocular drug delivery (diffusion rate etc)	
	Need to develop the plan for marketing plan with Dr. Bruce	Need to research more about different types of nanoparticles (at least three)	

**2/29/2016 A5 Log**

Experimental	Human Practice	Prototype	Research	Biobuilder Poster
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	Translated vet email and others.  Almost finished translating	Researched and found procedures for making nanoparticles		
	Aaron contacted glasses/contact lense manufacturer	Performed PCR check on GSR, could not read the labeling for CRYAA therefore could not predict the lanes.		
	Brainstormed ideas for spring fair games			

### **2/26/2015 B5 Log**

Experimental	Human Practice	Prototype	Research	Biobuilder Poster
	Vets Contacts research - we have 13 contacts found that are based both in Taiwan and USA (both vets, researchers, professors...)  Sent email to STATES ones already. (crossed out on contacts document = already sent out email)  Will send email after the Mandarin translation of email done.	Still need further research on eye drop	Research on 25 HC is on hold!	continued to work on images
	We sent out thank you cards to bioethics teachers (all 6)	reorganized nanoparticle research		will complete the poster (captions) by this weekend
		Colony PCR I		

### **2/25/2015 A5 Log**

CRYAA	Human Practice	Prototype	Treatment	Prevention
	Finalized questions for vets, wrote the final email to be sent to vets. DONE!	Found numerous links to more information about the eye drops	STILL NEED TO LOOK INTO SPECIFICS OF 25HC!	[Experimental] Created new plates (this update is to be confirmed)
	Aaron contacted an eye lens manufacturer. Created a list of questions for eye lens manufacturers.	Looked into "Chitosan nanoparticles for drug delivery to the eye" (a Review) - But need to conclude... - Updates about this soon		
	Created first draft of questions for people with cataracts (i.e. Dr. Moran, Mr. Clappers mother).			
	Created a variant set of questions for Dr. Wu (cataract surgeon + researcher)			

## 2/24/2015 B5 Log

CRYAA	Human Practice	Prototype	Treatment	Prevention
	Finished the human practice / outreach section for the poster.	Miniprep 10x His-Tag 4 Liquid Cultures -Concentration of Miniprep recorded on the lab notebook -All the parts for the project stored in the orange box named "counteract"		
	Worked on the email drafts for researchers/manufacturers. Both English and mandarin versions.	Did Transformation on CRYAA and GSR -GSR plates incubated overnight -CRYAA plates incubated overnight  Did Transformation on constitutive promoter + RBS -The cDNA is Bba_K880005 (from distribution kit 2015, plate 2 3F) : reconstituted with 10ul water Antibiotic: chloramphenicol -Refer to Lab notebook for more detail		

	Researched on pet cataracts: how often, how it happens, how it affects their daily life, how often/successful/common is surgery?	Autoclaved 50ml ddH2O to use for project experiments -Just making sure we have clean water for our experiments -LOL you go changyyy		
	ADDED PLAN: marketing strategy w/ Ms. Bruce!!!!	Researched on how nanoparticles send proteins into lenses. need to look more into it, and also how to ensure nanoparticles will stay in the lens		

### 2/23/2015 A5 Log

CRYAA	Human Practice	Prototype	Treatment	Prevention
	Researched organizations and veterinarians for dogs and cats.  Started making questions for animal organizations and veterinarians			
	Uploaded/completed Bioethics Panel video			
	Made pictures and graphics for the poster			
	Researched animal cataracts (in document labelled animal cataracts)			

### 2/22/2015 B5 Log

CRYAA	Human Practice	Prototype	Treatment	Prevention
	Bioethics final reminder emails final changes to questions and powerpoint practice go through intro and asking questions, also the introduction to our project	In the Prototype Google doc, We created a table to summarize the effectiveness of contact lenses, nanoparticles, and eye drops.		

	progress made on the 7th grade planning (worked both on the ppt and the plan)	Ran 10x his-tag on gel and the results were positive (336 bp)		
	updated FB cover photo~! YES THIS DESERVES TO BE ON HERE BECAUSE JASON IS REALLY PROUD OF HIS WORK :')	Ran PCR for CRYAA (All the primers and cDNA stored in a separate white box, called iGEM Experiment 2016)		
	continued to work on poster			

### 2/19/2015 A5 Log

CRYAA	Human Practice	Prototype	Treatment	Prevention
	<p><b>Created a powerpoint for the 7th grade lesson plan</b></p> <p>Further expanded on the current lesson plan (more work still needs to be done).</p> <p>Brainstormed more for fund raising ideas.</p>	<p>Performed PCR check on the transformed DNA today</p> <p>Also streaked 9 plates of 9 colonies and in the incubator</p> <p>Continued research on more effective ways to use the eyedrop. One way is to get the eyedrop in via the conjunctiva since it is 15% to 25% more permeable than the Cornea</p>		
	<p>Banner completed</p> <p>Worked more on graphics</p> <p>Created plan for video</p>	Decided to research on eyedrops for diseases that require it to reach further in the eye to see how they do it		
	Found more questions for doctors and organizations.			

### 2/18/2016 B5 log

CRYAA	Human Practice	Prototype	Treatment	Prevention
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	<p>BIOETHICS - virtually done, went over and revised the questions with Dr. Hsieh and deleted a few and made some changes.</p> <p><b>Made a powerpoint to present at the actual event - need finalized work on it. Austin will email Angela for the cataracts intro diagrams.</b></p>	<p>Further Researched into Chitosan and its relation to ocular delivery methods. (link at prototype folder)</p> <p>Designed Primers for GSR, Glutathione Reductase</p>		
	<p>Didn't get to do questions for organizations / public surveys (work on when panel is over)</p> <p>7th grade planning on hold!</p>	<p>Found compounds that make cornea easier to penetrate unsure about how safe these chemicals are though</p>		
	<p>Further worked on the biobuilder club poster (see austin for details) - <b>help needed with infographics and diagram!!</b></p>	<p>Transformed the 10x Histidine Tag Double Terminator from last year's distribution packet 2 (5p) into a NEW type of competent cells (labeled blue in the -80 storage) with NEW protocol for transformation</p> <p>The NEW protocol will be soon posted in the drive</p>		

## 2/17/2016 A5 log

CRYAA	Human Practice	Prototype	Treatment	Prevention
	<p>Edited the spring fair questions (added a new questions and refined old ones).</p> <p>Clarified schedule of bioethics and told Mr. Fagen he's off the hook</p>	<p>Trying to find a free vector mapping tool to better edit and view the construct we are trying to make</p> <p>Found Snap-Gene but free version is limited in capabilities</p> <ul style="list-style-type: none"> <li>30 day trial possible, will post link</li> </ul> <p>Dr. Chiang suggested using APE, a simply plasmid editor</p>	<p>Looked into how 25HC works!! found it.</p>	

	Worked on banner, outlined a design and began transferring it onto a digital form	Read a research paper on Chitosan Nanoparticles as a potential drug delivery paper, however the methods only were capable of reaching the cornea		
	Further worked on the biobuilder club poster (see austin for details)	Did research on dogs and cats cataracts as a potential subject for experimentation on		

## 2/16/2016 B5 log

CRYAA	Human Practice	Prototype	Treatment	Prevention
	<ul style="list-style-type: none"> <li>- Sent out bioethics panel final email (Mr. Maguire is now in place of Mr. Fagen!)</li> <li>- Some minor changes to the bioethics questions (they are sent to the teachers already!)</li> </ul>	<b>Delivery</b> <ul style="list-style-type: none"> <li>-Worked on Finding Nanoparticles for drug delivery in lenses.</li> <li>-Chitosan as a potential candidate</li> </ul>	<ul style="list-style-type: none"> <li>-found CYP27A1 and CH25H genes online</li> <li>-going to check the ORF frame</li> <li>-looked into INPNC -- a protein that can act as an anchor for proteins to attach to the cell</li> </ul>	
	<ul style="list-style-type: none"> <li>- looked over 7th grade plan but didn't make changes or add stuff</li> <li>- updated on questions for doctors</li> </ul>	<b>Construct</b> Candidates for Construct <ul style="list-style-type: none"> <li>- constitutive promoter</li> <li>- RBS</li> </ul> Still working on the best combination for producing greatest amount of Glutathione Reductase (GSR) - <b>refer to our itunes lab, 2-6 medium-strong combination works the best.</b> still need to test this though		



## 2/15/2016 A5 log

CRYAA	Human Practice	Prototype	Treatment	Prevention
	7th grade: -planning virtually completed	<b>I don't see this on the drive -Dr.Chiang</b>  Continued prototype planning  Added another step which was to potentially use protease to break off the His Tag after Immunoprecipitation  Did some research on nano-particles as a drug delivery option	<ul style="list-style-type: none"><li>- found compound 25hc</li><li>- found cholesterol → 25HC (oxidation)</li></ul>	Worked on experimental stuff
	Bioethics Panel: -finalized questions  Collab with 3D printer: -talk to Mr. Fagen about this (see WInnie)	Shared the Prototype Research on the IGEM 2016 Folder  Drew out a rough sketch of the constructs		
	Further developed questions for organizations (questions for organization doc)	Plan to start trying out the rough procedure to test viability with Bacterially Produced protein		

## 2/5/2016 B5 log

CRYAA	Human Practice	Prototype	Treatment	Prevention
	<ul style="list-style-type: none"><li>- Worked on Biobuilder poster<ul style="list-style-type: none"><li>- Infographic (That may or may not be used on the poster's</li><li>- Other diagrams (i.e. lens diagram)</li></ul></li><li>- We'll need to work on the Biobuilder Abstract &amp; other sections of the poster</li></ul>			
	<ul style="list-style-type: none"><li>- Finalized questions for doctors</li><li>- So far there are 4 doctors that will be interviewed (Steph's dad, Avery's dad, and 2 doctors from Chang Gung Hospital at Linkou</li></ul>	-		

## 2/4/2016 A5 log

CRYAA	Human Practice	Prototype	Treatment	Prevention
	<ul style="list-style-type: none"> <li>- Finished editing the bioethics panel questions</li> <li>- Worked on poster</li> </ul>	<ul style="list-style-type: none"> <li>- Started planning out what to do for After CNY considering experiments</li> <li>- Going to try test out the protocol using the 10x-Hist Tag given by IGEM</li> <li>- Planned out a general flow of how to approach the experiment</li> <li>- attempted to design a construct for GSHX/GSHR/CRYAA</li> </ul>	<p>→ Treatment needs the most help</p> <p>→ Found out that Lanosterol is involved in a (generalized) 3 step pathway: Squalene makes Lanosterol, which in turn can make cholesterol → We need to dig deeper into the Lanosterol → Chol. pathway, and <b>find enzymes involved</b> to try to inhibit it.</p>	<p>→ Found info about how Connexin &amp; Aquaporin play a role in the formation of the barrier</p> <p>→</p>
	<ul style="list-style-type: none"> <li>- Added questions to student survey</li> <li>- Developed questions for Avery and Stefanie's parents</li> </ul>	<ul style="list-style-type: none"> <li>- Already a backbone along with the 10x-Hist Tag pSB613 so potentially able to use that as our backbone for all the constructs</li> </ul>		

## 2/3/2016 B5 log

CRYAA	Human Practice	Prototype	Treatment	Prevention
<p>Mapped sequence</p> <p>Resolved a problem regarding the disadvantage of CRYAA</p>	<p><b>Keep working on the bioethics questions!!!!</b></p> <p>Done some FB changes :) GO LIKE THE PAGE AND POSTS GUYS!</p> <p>Doc created for poster outline</p> <p>Questions for Steph &amp; Avery to interview their</p>	<p>Significant progress</p> <ul style="list-style-type: none"> <li>- found protocols for Bacterial protein purification using Hist tag</li> <li>- Justin found a protocol ask him for more details</li> </ul>	<p>Refer to the diagram (drawn on a paper in the lab) on the squalene→ lanosterol → cholesterol</p>	<p>Oxidation</p> <ul style="list-style-type: none"> <li>-Updated diagram</li> <li>-Check diagram to get updated / review for the entire mechanism</li> </ul>

	<p>Dads (which will used for other doctors too)</p> <p>Survey questions need revision! (opinions &gt; experiences because we can get stats elsewhere, more on questions that can influence/change our prototype focus)</p> <p>GO FUND ME (?) we need some charity / fundraising ideas</p>			
	<p>Worked on creating a cataracts survey to get public opinion</p> <p>-</p>	<p>- Justin found a contact lenses</p>	<p>Need to find the particular pathway from Lanosterol to cholesterol → Because we want to see if we can reverse cholesterol back to lanosterol (which is helpful)</p> <p>We also need to fine a way to stimulate more of: squalene → lanosterol</p>	<p>Figured out what constitutes the "barrier" -- Aquaporin0, Conexin</p> <p>Now we need to find out more information about 1) what the two proteins are exactly, and 2) the pathways by which AQP0 and cxn actually degraded</p>

## 2/2/2016 A5 log

CRYAA	Human Practice	Prototype	Treatment	Prevention
<p>Found more info about the chemical structure of alpha crystallin protein</p> <ul style="list-style-type: none"> <li>- N-C terminal diagram in the lab</li> <li>-</li> </ul>	<p>Further finalized and cleaned up the questions for the bioethics panel</p>	<p>Significant progress</p> <ul style="list-style-type: none"> <li>- found protocols for Bacterial protein purification using Hist tag</li> <li>- Justin found a protocol ask him for more details</li> </ul>	<p>Refer to the diagram (drawn on a paper in the lab) on the squalene→ lanosterol → cholesterol</p>	<p>Oxidation</p> <ul style="list-style-type: none"> <li>-Updated diagram</li> <li>-Check diagram to get updated / review for the entire mechanism</li> </ul>

<p>Questions that still need to be answered:</p> <ul style="list-style-type: none"> <li>- Too much alpha-crys apparently leads to neurodisease? (confirm this)</li> <li>- Why?</li> <li>- Then what happens if too little alpha crys? and How?</li> </ul>	<p>Worked on creating a cataracts survey to get public opinion</p> <ul style="list-style-type: none"> <li>-</li> </ul>	<ul style="list-style-type: none"> <li>- Justin found a contact lenses</li> </ul>	<p>Need to find the particular pathway from Lanosterol to cholesterol → Because we want to see if we can reverse cholesterol back to lanosterol (which is helpful)</p> <p>We also need to fine a way to stimulate more of: squalene → lanosterol</p>	<p>Figured out what constitutes the "barrier" -- Aquaporin0, Connexin</p> <p>Now we need to find out more information about 1) what the two proteins are exactly, and 2) the pathways by which AQP0 and cxn actually degraded</p>
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Slogans:

- counteracts
- non-invasive cataract solutions
- cataract killer
- preventing and treating cataract formation
- make your lens clear again
- cataract kungfu
- to see or not to see
- oh say can you see

**2/1/2016 B5 log**

CRYAA	Human Practice	Prototype	Treatment	Prevention
<p>Found more info about the chemical structure of alpha crystallin protein</p> <ul style="list-style-type: none"> <li>- N-C terminal diagram in the lab</li> <li>-</li> </ul>	<p>Finalized List of teachers for Bioethics Panel</p>	<p>No significant progress :( See below for what we have so far</p>	<p>Refer to the diagram (drawn on a paper in the lab) on the squalene→ lanosterol → cholesterol</p>	<p>Oxidation</p> <ul style="list-style-type: none"> <li>-Updated diagram</li> <li>-Check diagram to get updated / review for the entire mechanism</li> </ul>
<p>Questions that still need to be answered:</p> <ul style="list-style-type: none"> <li>- Too much alpha-crys apparently leads to neurodisease? (confirm this)</li> <li>- Why?</li> <li>- Then what happens if too little alpha crys? and How?</li> </ul>	<p>Bioethics Panel:</p> <ul style="list-style-type: none"> <li>- Completed the questions</li> <li>- Finalized list of teachers</li> <li>- Dr. Smith</li> <li>- Dr. Vidal</li> <li>- Dr. Costello</li> <li>- Mr. Anderson</li> <li>- Mr. Fagen</li> <li>- Ms. Vinod (? waiting for response</li> <li>- Mr. Montgomery</li> </ul>	<p>Eye drops</p> <ul style="list-style-type: none"> <li>- What are the composition s of eye drops?</li> <li>- What do we need (in addition to the proteins that we're going to create)?</li> </ul>	<p>Need to find the particular pathway from Lanosterol to cholesterol → Because we want to see if we can reverse cholesterol back to lanosterol (which is helpful)</p> <p>We also need to fine a way to stimulate more of: squalene → lanosterol</p>	<p>Figured out what constitutes the "barrier" -- Aquaporin0, Connexin</p> <p>Now we need to find out more information about 1) what the two proteins are exactly, and 2) the pathways by which AQP0 and cxn actually degraded</p>

**1/29/2016 A5 log**

CRYAA	Human Practice	Prototype	Treatment	Prevention
(Consult Leon & Austin)	Further Brainstormed the plan for 8th grade research.	(Consult Hansen, Justin, or Christy)	Lanoterol,, Antioxidants New Methods Order	Oxidation -Updated diagram
(Lucy) Researched on Chaperoning function & oxidation effects - Found	Bioethics Panel: - Worked further on developing questions - Added a few potential teachers to our options - For now we're going to wait on responses from our current people before we add additional teachers	Contact Lenses		- Lens barrier: Still need to look more into the Lens barrier vs. O2 availability relationship - Mitochondria vs. Age - Order
		Eyedrops		
		Immunoprecipitation Kit - Found more kits sold separately averaging the price - Consulted Kevin about the potential problems of using IP Kits for Mammalian cells for Bacteria Cells - Potential way of purifying protein from the Bacteria then start IP from there <b>-Pulldown</b> another procedure is a potential alternate-procedure -Justin and I will ask our respective labs about IP to save time on research -Found procedure for purifying protein from Bacteria, seems viable to continue IP from the purified protein at the end of the Bacteria Purifying Procedure		

### 1/28/2016 B5 log

Experiment	Research	Human Practice	Miscellaneous
cloning cycle	<b>shift focus → BARRIER:</b> - what is the barrier? - why does the barrier form? -composition of the barrier? -why does mitochondria activity decrease as you age?	Bioethics Panel <ul style="list-style-type: none"> <li>email more teachers?</li> <li>QUESTIONS!!!!</li> </ul>	
	<b>Things to still focus on</b> -other treatments? -relationship between age and lanosterol? -other factors that contribute to cataracts - Final product - Prx? eye drops? eye contacts	March - April <ul style="list-style-type: none"> <li>4 sessions</li> <li>w/ 7th grade</li> </ul>	solidify understanding of formation of cataracts
	<b>Things that are on hold about truncation:</b> -CRYAA prevent protein clumping with hydrophobic / hydrophilic regions on the protein <b><u>still need to find the exact regions that are preventing clumping</u></b> -How increase in Ca results in Calpain activity -what truncates CryAA		CRYAA gene ordered. should come soon
	<b>Diabetes (on hold too)</b> -High glucose level can activate the polyol pathway or lead to glycation -polyol pathway converts glucose into sorbitol and then into fructose -glycation is the spontaneous binding of protein with sugar		

### 1/26/2016 B5 log

Experiment	Research	Human Practice	Miscellaneous
cloning cycle	Questions to answer: <ul style="list-style-type: none"> <li>How increase in Ca results in Calpain activity</li> <li>what truncates CryAA</li> <li>relationship between squalene and age?</li> </ul>	made progress for thursday kindergarten stuff	keep researching

	<ul style="list-style-type: none"> <li>• other factors that contribute to cataracts</li> <li>• Prx? cataracts? eye drops?</li> </ul>		
	<p>Found diabetes as an additional factor that contribute to cataracts</p> <ul style="list-style-type: none"> <li>- High glucose level can activate the polyol pathway or lead to glycation</li> <li>- polyol pathway converts glucose into sorbitol and then into fructose</li> <li>- glycation is the spontaneous binding of protein with sugar</li> </ul>		solidify understanding of formation of cataracts
	<p>CRYAA prevent protein clumping with hydrophobic / hydrophilic regions on the protein</p> <p><b><u>still need to find the exact regions that are preventing clumping</u></b></p>		CRYAA gene ordered. should come soon
	<p>squalene lss relationship established</p> <p><b><u>find age lanosterol link</u></b></p>		

**1/25/2016 A5 log**

Experiment	Research	Human Practice	Miscellaneous
cloning cycle stuff	<p>Questions to answer:</p> <ul style="list-style-type: none"> <li>• How increase in Ca results in Calpain activity</li> <li>• what truncates CryAA</li> <li>• relationship between squalene and age?</li> <li>• other factors that contribute to cataracts</li> </ul>	made progress for thursday kindergarten stuff	keep researching

	<ul style="list-style-type: none"> <li>• Prx? cataracts? eye drops?</li> </ul>		
tested kindergarten soap milk experiment	Calpain: two binding sites, calcium acts as catalyst /cofactor and enhances calpain activity		solidify understanding of formation of cataracts
	Found truncation and CRYAA by calpain		CRYAA gene ordered. should come soon
	CRYAA prevent protein clumping with hydrophobic / hydrophilic regions on the protein <b><u>still need to find the exact regions that are preventing clumping</u></b>		spend some time thinking about what we want to do with CRYAA gene next week
	squalene lss relationship established <b><u>find age lanosterol link</u></b>		
	found a ton of ordering sites for lss, squalene, and cryaa		

#### 1/22/2016 B5 log

Experiment	Research	Human Practice	Miscellaneous
Cloning	Specific amino acid truncated from alpha crystallin	Kindergarten (1/28, B2)  Done: <ul style="list-style-type: none"> <li>• Found 5 potential interactive topics</li> </ul> <b>Needs to be done:</b> <ul style="list-style-type: none"> <li>• Try out different experiments</li> <li>• begin writing concrete lesson plan</li> <li>• Gathering materials once lesson plan is completed</li> </ul>	Sizes <ul style="list-style-type: none"> <li>- XS</li> <li>- S</li> <li>- M</li> <li>- L</li> <li>- XL</li> </ul>
Cleaning	age and lanosterol correlation (kinda sad)... New direction: age and squalene relation. Other	Bioethics Panel  Done:	



	possible treatment routes	<ul style="list-style-type: none"> <li>email sent out</li> </ul> Needs to be done: <ul style="list-style-type: none"> <li>Still need to develop questions and topics of discussion</li> </ul>	
	finding price for squalene synthase and cDNA. currently working on: ordering squalene (steroid) ^^for lanosterol	8th Grade teaching  needs to be done: <ul style="list-style-type: none"> <li>Email to Mr. Brocklehurst needs to be drafted</li> <li>Developing Syn Bio focused lesson and interactive labs</li> <li>brainstorming ideas</li> </ul>	
	relationship of cataracts and dog/cats - animal model	Find experts who are also working on cataracts	
	other things to research on: what truncations give the worse disease? how does the chaperone activity work?		

#### 1/21/2016 A5 log

Experiment	Research	Human Practice	Miscellaneous
Cloning	Specific amino acid truncated from alpha crystallin	<b>Kindergarten (1/28, B2)</b>  Done: <ul style="list-style-type: none"> <li>Found 5 potential interactive topics</li> </ul> <b>Needs to be done:</b> <ul style="list-style-type: none"> <li>Try out different experiments</li> <li>begin writing concrete lesson plan</li> <li>Gathering materials once lesson plan is completed</li> </ul>	Sizes - XS - S - M - L - XL
Cleaning	age and lanosterol correlation (kinda sad)... New direction: age and squalene relation.	Bioethics Panel  Done: <ul style="list-style-type: none"> <li>email sent out</li> </ul> Needs to be done: <ul style="list-style-type: none"> <li>Still need to develop questions and topics of</li> </ul>	

		discussion	
	finding price for squalene synthase and cDNA. currently working on: ordering squalene (steroid) ^^for lanosterol	8th Grade teaching  needs to be done: <ul style="list-style-type: none"> <li>Email to Mr. Brocklehurst needs to be drafted</li> <li>Developing Syn Bio focused lesson and interactive labs</li> <li>brainstorming ideas</li> </ul>	
	focus on: other treatment routes,		

#### 1/20/2016 B5 log

Experiment	Research	Human Practice	Miscellaneous
Cloning	PRIORITY age and lanosterol <b>(NEED TO WORK MORE ON THIS)</b>	Bioethics Panel Email <b>(B5 - done with draft, A day please confirm with Mr. Clapper + make final changes and send out. If sent on Thursday someone tell Angela)</b>	Sizes <ul style="list-style-type: none"> <li>- XS</li> <li>- S</li> <li>- M</li> <li>- L</li> <li>- XL</li> </ul>
Cleaning	Look up genes other than CRYAA <b>(added some vendors)</b>	Organizations Doctors <b>(still need to look up)</b>	
	Causes <b>(in progress)</b>		
	Symptoms		

#### 1/19/2016 A5 To-Do List:

Experiment	Research	Human Practice	Miscellaneous
Cloning	PRIORITY Link v/w age and lanosterol	Kindergarten (1/28, B2)	Sizes <ul style="list-style-type: none"> <li>- XS</li> <li>- S</li> <li>- M</li> <li>- L</li> <li>- XL</li> </ul>

Cleaning	Look up genes other than CRYAA	Bioethics Panel (email by end of week)	
new DNA agarose gels (+seeing safe)	Causes	8th Grade? - teach synbio	
	Symptoms	Doctors? Organizations? Other HP activities?	
	PRIORITY Ordering Info look up cDNA for CRYAA		

**1/18/2016 B5 To-Do List:**

Experiment	Research	Human Practice	Miscellaneous
Cloning	Look up relevant genes	Bioethics Panel	T-Shirt/Sweatshirt -- survey for size
Cleaning	Lookup cDNA to buy	Compile pics + stuff from Kindergarten	New Logo (for the Cataracts idea)
Bio Waste	What's on parts registry?	Plan for 8th grade	
	What to model w/ computer?	Stats & figures on cataracts	
	Lanosterol & age -- correlation & effects?	Look up and seek organizations, doctors, etc.	